Published online May 18, 2006

SSR Markers Exhibit Trisomic Segregation Distortion in Soybean

J. J. Zou,* R. J. Singh, J. Lee, S. J. Xu, and T. Hymowitz

ABSTRACT

A primary trisomic ($2n = 2x + 1$) is an excellent cytogenetic tool to locate genes and associate linkage groups to their respective chromosomes in diploid plant species. In soybean [*Glycine max* (L.) Merr.], 11 molecular linkage groups (MLGs) were previously assigned to their respective chromosomes using simple sequence repeat (SSR) markers and primary trisomics. The chromosome location of the SSR markers was determined by altered segregation ratios from a disomic (1:2:1) to a trisomic genotypic ratio (6:11:1) in the F_2 offspring derived from the crosses of soybean primary trisomics and *G. soja* Siebold & Zucc. In this study, we established the association between soybean Triplo 4 and MLG C1 based on trisomic segregation ratio (6:11:1) and cytological analysis. In addition, we identified four SSR markers that exhibited trisomic segregation distortion on three soybean chromosomes. Those SSR markers, including Satt565 and SOYGPATR on chromosome 4 (MLG C1), Satt193 on chromosome 13 (MLG F), and Satt226 on chromosome 17 (MLG D2) segregated in a 1:11:6 ratio, which is the reverse of the standard trisomic genotypic ratio. This is the first report of trisomic segregation distortion in soybean. Since *G. max* and *G. soja* usually differ either by a reciprocal translocation or by a paracentric inversion, we hypothesize that the genomic divergence between the two species and the numerically unbalanced genome in the trisomics might contribute to the distorted trisomic ratios. Genome instability may have been triggered in F_1 primary trisomics causing unexpected trisomic inheritance in F_2. Our results indicate that both normal and distorted trisomic segregation ratios should be considered when analyzing the association between chromosomes and linkage groups using primary trisomic analysis.

An individual with a normal chromosome complement plus an extra complete chromosome ($2n = 2x + 1$) is known as a primary trisomic, and the individual is called Triplo (Singh, 2003). Since the classical studies of *Datura* trisomics by Blakeslee (Blakeslee, 1921), primary trisomics have been used extensively to associate marker genes with a particular chromosome, to associate a genetic linkage group with the individual chromosome, and to test the independence of linkage groups (Singh, 2003). The cytogenetic maps in maize (*Zea mays* L.) (Rhoades and McClintock, 1935), tomato (*Lycopersicon esculentum* Mill.) (Rick and Barton, 1954), barley (*Hordeum vulgare* L.) (Tsuchiya, 1967), and rice (*Oryza sativa* L.) (Khush et al., 1984) have been established utilizing the primary trisomic method.

J.J. Zou, Pioneer Hi-Bred International, Inc., Johnston, IA 50131; S.J. Xu, USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58105; J. Lee, Genetic Resources Division, National Institute of Agricultural Biotechnology, Suweon, 441-701, South Korea; R.J. Singh and T. Hymowitz, Department of Crop Sciences, University of Illinois, Urbana, IL 61801. Received 22 July 2004. *Corresponding author (Jijun.Zou@pioneer.com).

Published in Crop Sci. 46:1456–1461 (2006).
Crop Breeding & Genetics
doi:10.2135/cropsci2004.0451

677 S. Segoe Rd., Madison, WI 53711 USA

The association of a gene to a specific chromosome can be determined by the modified segregation ratios in the F_2 offspring of primary trisomics. Genetic ratios are modified from the expected disomic ratio (3:1, dominant; 1:2:1, codominant) to a trisomic ratio (17:1, dominant; 6:11:1, codominant) if the female transmission rate of the extra complete chromosome is 50%, and assuming no male transmission of $n + 1$ spores. Sometimes, the female transmission rate of the extra chromosome is 33.3%, in which case the expected trisomic ratios are 12.5:1 (dominant) or 5:7.5:1 (codominant) (Singh, 2003).

A set of primary trisomics have been identified in soybean (Xu et al., 2000), and have been used to associate morphological mutants and SSR markers to their respective chromosomes. Honeycutt et al. (1990) associated a variegated leaf mutant gene (*v2*) with chromosome 5. Hedges and Palmer (1991) placed an isozyme marker, *dia1* (diaphorase), on chromosome 4. Xu et al. (2000) associated two seed protein genes *eu1* (urease), *lx1* (lipoxygenase), and a morphological marker gene *p2* (puberulent) with chromosomes 5, 13, and 20, respectively. Gardner et al. (2001) and Zou et al. (2003a) associated *Rps1-k* (resistant to phytophthora root rot) and *y10* (yellow leaf mutant) with chromosome 3, respectively. Using SSR markers and primary trisomics, eleven molecular linkage groups (MLGs) have been assigned to their respective chromosomes (Zou et al., 2003b). However, there are still large numbers of uncharacterized soybean primary trisomics, and their associations with linkage groups are unknown.

Segregation distortion has been widely reported in different plant species, such as tomato (Paterson et al., 1988), barley (Heun et al., 1991), rice (Xu et al., 1997), and soybean (Webb et al., 1995; Yamanaka et al., 2001). However, most of the reports were based on disomic segregation, and there was no report on distorted trisomic segregation. During our work to assign linkage groups to soybean chromosomes, we have observed SSR markers from three chromosomes that showed distorted trisomic segregation, which is the reverse of the standard trisomic genotypic ratio. The main objective of this paper is to report the association between MLG C1 and soybean chromosome 4, and the observed trisomic segregation distortion.

MATERIALS AND METHODS

Plant Material and Cytological Analysis

Primary trisomics in soybean were isolated and identified (Triplo 1 through Triplo 20) in the genetic background of soybean cv. Clark 63 (Xu et al., 2000). A few other primary trisomics that were not accurately identified were also included in this study. Each primary trisomic was crossed with *Glycine*

Abbreviations: MLG, molecular linkage group; SSR, simple sequence repeat; QTL, quantitative trait loci.

soja accessions PI 407287 or PI 81762 to provide a high degree of molecular polymorphism. F_1 plants with $2n = 40$ and 41 chromosomes were identified cytologically (Xu et al., 2000). After cytological analysis, the F_1 hybrids with $2n = 40$ (one plant as a control) and $2n = 41$ (at least 2 plants) were grown in the greenhouse and allowed to produce selfed seeds. Fifty to 100 F_2 seeds from the disomic ($2n = 40$ F_1 plant)- and trisomic ($2n = 41$ F_1 plant)-derived populations were germinated in the greenhouse, and DNA was isolated from young leaves of each plant using the method of Walbot (1988).

Primary trisomics in soybean can be verified by meiotic pairing analysis of the F_1 plants with $2n = 42$ derived from crosses between two primary trisomics since the extra chromosomes of the trisomics can be transmitted through pollen (Palmer, 1976; Xu et al., 2000). We noticed that some primary trisomics exhibited similar morphological alterations in plant stature and size of leaflets and pods. To test the potential duplication of primary trisomics, selected trisomics were hybridized with each other. All the F_1 plants with $2n = 42$ were identified cytologically and were grown in the greenhouse. Chromosome pairing was examined at meiotic metaphase-I.

SSR Marker Analysis

The isolation and map positions of soybean SSR markers have been described by Cregan et al. (1999). PCR reactions were undertaken in 10-ul volumes containing 30 to 45 ng of template DNA, 1.5 pmole of each primer, 0.2 m*M* dNTPs (Pharmacia Biotech Inc., Piscataway, NJ), 1.5 m*M* $MgCl_2$, 1× PCR buffer and 0.5 unit *Taq* polymerase (Gibco BRL Life Technologies Inc., Gaithersberg, MD). Temperature cycling was performed in an MJ Research PTC 100 Thermal Controller using 'touchdown' PCR. The amplification profile was set to run at 94°C for 3 min followed by 6 cycles of denaturing at 94°C for 30 sec, annealing from 55 to 50°C with 1°C decreased by one cycle for 30 s, and extending at 72°C for 1 min. The final cycle (94°C for 30 sec, 50°C for 30 s, and 72°C for 1 min) was repeated 35 times. Amplification products were detected by 6% (w/v) denaturing polyacrylamide gel electrophoresis and silver staining.

Test of Segregation Ratio

Chi-square analysis was performed with MS Excel to test if the segregation ratios of SSR markers fit the disomic ratio (1:2:1), a trisomic ratio (6:11:1), or a reverse trisomic ratio (1:11:6), assuming a 50% female transmission rate of the extra chromosome in the progeny of primary trisomics.

RESULTS

To establish the association between linkage groups and soybean chromosomes we are continuing to examine primary trisomics-derived F_2 populations using SSR markers. Triplo 4 was one of the earliest identified soybean primary trisomics, but its association with soybean chromosome was unknown. An F_2 population from Triplo 4 × PI 407287 ($2n = 40$) was initially examined with SSR markers from all the 20 MLGs, but none of the SSR markers had the expected trisomic ratio (6:11:1) (Table 1). Among these markers, Satt565 (from top of MLG C1) displayed a 2:11:13 ratio, which did not fit a disomic ratio (1:2:1). When we increased the F_2 population size, it segregated in a ratio of 5:46:34. Another SSR marker, SOYGPATR that is closely linked to Satt565 (MLG C1), also displayed a similar ratio of 5:47:30 (Table 2). By contrast, Sat_085 and Satt180, located in the middle and bottom of MLG C1, respectively, showed a 6:11:1 ratio (Table 2; F_1–1-derived F_2 population in Fig. 1), suggesting association of MLG C1 with chromosome 4. We tested each of the four markers in another population of Triplo 4 × PI 407287 (F_1–2-derived F_2 population in Fig. 1). Each marker segregated in trisomic fashion, confirming that MLG C1 is located on chromosome 4. As an example, PCR amplification results of Satt565 in the two Triplo 4 F_1-derived F_2 populations are shown in Fig. 2. Thus, Satt565 and SOYGPATR, exhibited a distorted trisomic ratio in one population and a trisomic ratio in another population. We also examined the segregation data in a disomic F_1 ($2n = 40$)-derived population (F_1–3-derived F_2 popula-

Table 1. SSR markers used in the initial analysis of F_2 population derived from trisomic F_1 plants from cross of Triplo 4 and PI 407287, segregation ratios, and chi-square probability for goodness of fit to 1:2:1 and 6:11:1 ratio.

SSR loci	Linkage group	F_2 segregation ratio	Chi-square probability	
			1:2:1	6:11:1
Satt236	A1	5:11:6	0.96	<0.01
Satt329	A2	9:29:14	0.44	<0.01
Satt197	B1	9:11:6	0.52	<0.01
Satt534	B2	5:10:9	0.37	<0.01
Satt565	C1	2:11:13	<0.01	<0.01
Sat_076	C2	7:10:9	0.43	<0.01
Satt184	D1a+q	6:12:8	0.79	<0.01
Satt141	D1b+w	6:14:5	0.80	<0.01
Satt311	D2	13:33:10	0.35	<0.01
Satt411	E	5:12:5	0.91	<0.01
Satt252	F	4:9:7	0.58	<0.01
Satt566	G	9:7:8	0.12	<0.01
Satt302	H	5:13:10	0.38	<0.01
Satt367	I	10:11:7	0.38	<0.01
Satt596	J	6:15:5	0.71	<0.01
Satt381	K	15:23:12	0.71	<0.01
Satt143	L	5:13:8	0.71	<0.01
Satt536	M	16:28:9	0.36	<0.01
Satt091	N	6:11:9	0.52	<0.01
Satt173	O	8:11:6	0.71	<0.01

Table 2. SSR markers used in different populations, F_2 segregation, and chi-square probability for goodness of fit to 1:2:1, 6:11:1, and 1:11:6 ratios.

SSR locus	F_2 population†			Chi-square probability‡		
	T	F	S	1:2:1	6:11:1	1:11:6
MLG C1– Triplo 4 × PI 407287						
Satt565	5	46	34	<0.01	<0.01	0.40
SOYGPATR	5	47	30	<0.01	<0.01	0.78
Sat_085	26	49	5	<0.01	0.96	
Satt180	25	48	5	<0.01	0.93	
MLG D2– 97UT144 × PI 407287						
Satt154	19	20	3	<0.01	0.20	
Satt226	3	39	16	<0.01	<0.01	0.62
Satt543	11	12	1	<0.05	0.43	
Sat_114	9	12	1	<0.05	0.75	
Satt514	9	14	1	<0.05	0.89	
Satt311	25	47	5	<0.01	0.93	
Sct_137	15	22	3	<0.05	0.70	
MLG F– Triplo 13 × PI 407287						
Satt193	4	57	33	<0.01	<0.01	0.83
Satt252	15	30	4	<0.05	0.70	
Satt114	22	72	2	<0.01	0.14[b]	
Satt510	22	23	2	<0.01	0.15	
Satt171	7	13	0	<0.05	0.56	

† T, Triplo; F, F_1, and S, *G. soja*.
‡ Chi-square test is for (T + F):S against 17:1 ratio.

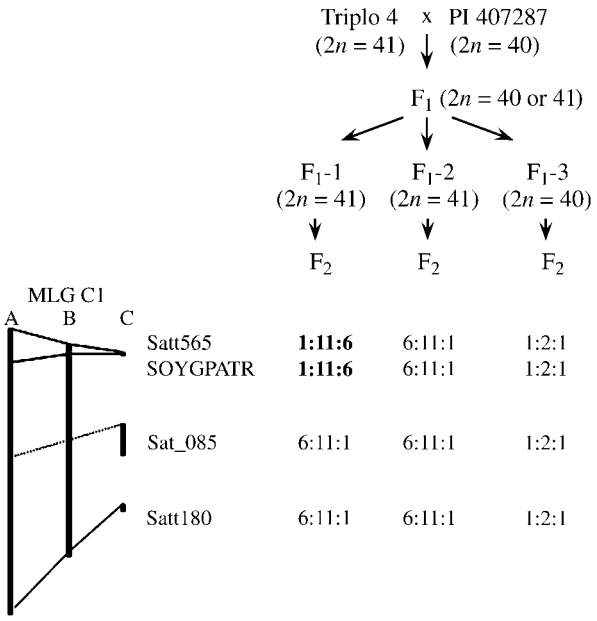


Fig. 1. Segregation ratios of SSR markers in different F_2 populations. The linkage groups were according to Cregan et al. (1999). Linkage groups A, B, and C were developed by USDA/Iowa State Univ., the Univ. of Utah, and the Univ. of Nebraska, respectively. Segregation ratios were based on marker classes of Triplo 4: F_1: PI 407287.

tion in Fig. 1). All markers segregated in normal disomic fashion. MLG C1 has been associated with Triplo 14 using SSR markers, and all markers displayed a 6:11:1 ratio (Zou et al., 2003b). It can be inferred that Triplo 4 and Triplo 14 carried the same extra chromosome.

Genetic stock 97UT144 ($2n = 41$) was identified as a primary trisomic, but hasn't been associated with any linkage group. SSR markers from 20 MLGs were initially examined in an F_2 population of 97UT144 × PI 407287. All markers segregated in a disomic ratio except that Satt226 (MLG D2) showed a 0:17:4 ratio, which did not fit either a disomic or a trisomic ratio. When we increased the F_2 population size, it segregated in a 3:39:16 ratio. Six additional loci (Satt154, Satt543, Sat_114, Satt514, Satt311, and Sat_137) from MLG D2 were tested with the same population, and they all segregated in trisomic fashion (Table 2). These results suggest that MLG D2 is located on the extra chromosome of 97UT144. MLG D2 has been associated with Triplo 17 in the previous study because Satt226, along with Sctt008, Satt311, and Sct_137, all segregated in a 6:11:1 fashion (Zou et al., 2003b). This indicates 97UT144 and Triplo 17 carry the same extra chromosome. A cross was further made between 97UT144 and Triplo 17. The identity of those two trisomics was confirmed by the meiotic chromosome pairing in the hybrids.

MLG F has been assigned to chromosome 13 using a trisomic-derived F_2 population (Cregan et al., 2001). When we examined SSR markers in another Triplo 13-derived population (Triplo 13 × PI 407287), we found Satt193 (MLG F) displayed a distorted ratio (4:57:33), while other markers from this linkage group showed a trisomic ratio (Table 2). The SSR markers that are closely linked with Satt193 did not exhibit polymorphism between Triplo 13 and PI 407287, so we were unable to examine those marker-segregation ratios.

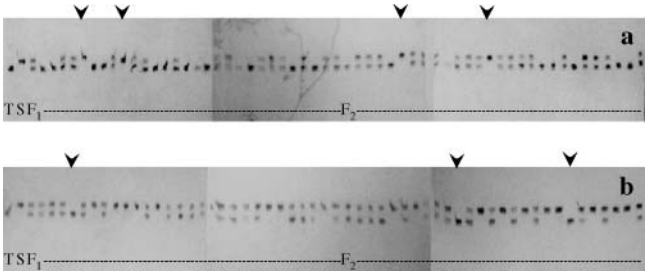


Fig. 2. a, b SSR pattern of trisomic derived F_2 individuals amplified using Satt565. T: Triplo 4; S: *G. soja* PI 407218; F_1: F_1 hybrid; Arrows point to the specific F_2 plant that was homozygous for the least frequent homozygous marker class. a SSR marker analysis of 57 F_2 plants from a Triplo 4 F_1 hybrid (F_1–2 in Fig. 1). *G. soja* PI 407287 marker class was least represented in F_2 population. b SSR marker analysis of 55 F_2 plants from a Triplo 4 F_1 hybrid (F_1–1 in Fig. 1). Triplo 4 marker class was least represented in F_2 population.

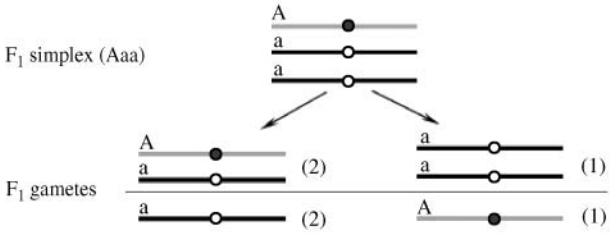
DISCUSSION

The associations between the linkage groups and soybean chromosomes will help to better understand the structure of the soybean genome and the physical organization of the genes in the genome. Primary trisomics are an invaluable tool to establish such associations. In the current study, we have associated MLG C1 with Triplo 4 using SSR markers and trisomic-derived F_2 segregation populations. Triplo 4 was originally named Tri D, which was one of the earliest five primary trisomics characterized and arbitrarily designated in soybean (Palmer, 1976; Hedges and Palmer, 1991). Hedges and Palmer (1991) located an isozyme marker, *dia 1* (diaphorase), on the extra chromosome of Tri D by using starch gel electrophoresis. Now we can assign *dia 1* to linkage group C1 based on the established association between linkage group and soybean chromosome. Most of the soybean primary trisomics were morphologically indistinguishable. It is labor intensive and time consuming to determine if the trisomics carry the same extra chromosome. Traditionally, we have to make crosses among all the available trisomics to examine the possible duplications. Using molecular markers, we can quickly screen the candidate primary trisomics in their derived segregation populations. For example, we have identified the duplications of Triplo 4/Triplo 14 and 97UT144/Triplo 17 with the assistance of SSR markers.

Generally, we considered the trisomic segregation ratio as 6 (trisomic type):11 (F_1 type):1 (disomic type) based on 50% female transmission rate. In this study, we found that SSR markers from three soybean chromosomes showed distorted trisomic segregation ratio. Those SSR markers segregated in a 1:11:6 ratio, which is the reverse of the standard trisomic genotypic ratio. Meanwhile, the markers from other parts of the same linkage group showed normal trisomic segregation. One may postulate that change has occurred from duplex (AAa) to simplex (Aaa) genotype during gametogenesis in an F_1 plant. Assuming random chromosome segregation, the expected frequencies of gametic types from

a primary trisomic F_1 of duplex (AAa) genotypic constitution with 50% female transmission of the extra chromosome are as follows: $(n + 1)$, 2Aa + 1AA and n, 2A + 1a. The expected genotypic frequencies in F_2 are as follows: $2x + 1$ = 2AAA + 5AAa + 2Aaa and $2x$ = 4AA + 4Aa + 1aa. The total ratio will be 6 (2AAA+ 4AA): 11 (5AAa+2Aaa+4Aa): 1 (1aa). If a trisomic locus was changed to a disomic locus, the genotype of a primary trisomic F_1 would be changed from duplex (AAa) to simplex (Aaa). The expected frequencies of gametic types from a simplex (Aaa) F_1 genotype are: $(n + 1)$, 2Aa + 1aa and n, 1A + 2a (Fig. 3). The expected genotypic frequencies in the F_2 are as follows: $2x + 1$ = 2AAa + 5Aaa + 2aaa and $2x$ = 1AA + 4Aa + 4aa. The total F_2 segregation ratio will be 1(1AA): 11 (2AAa+ 4Aa+5Aaa): 6 (2aaa+4aa). Thus, the change of a trisomic locus to a disomic locus could account for the increased ratio of disomic locus (1:11:6 instead of 6:11:1 ratio in the F_2 populations). However, the chance of a mutation is rare and further studies are needed to examine the possible locus change in the F_1 trisomic plants.

Distorted segregation ratios of molecular markers are often observed in progeny derived from inter- and intraspecific crosses and they may be caused by competition among gametes for preferential fertilization or from abortion of the gamete or zygote (Faris et al., 1998; Lyttle, 1991). In our study, the F_2 populations were all derived from the interspecific hybrids between primary trisomics of cultivated soybean *G. max* and its wild progenitor *G. soja.* Several cytogenetic studies have demonstrated that *G. max* and *G. soja* carry the same GG genomes, but the two species usually differ by a reciprocal translocation (Hadley and Hymowitz, 1973; Palmer et al., 1987; Singh and Hymowitz, 1988). Palmer et al. (1987) reported that 46 of 56 *G. soja* accessions from China and the former Soviet Union contained



F_2 genotypes (50% female transmission rate)

♀ \ ♂	n 1A	2a	$n+1$ 2Aa	1aa
$n+1$ 2Aa	2AAa	4Aaa	No transmission	
$n+1$ 1aa	1Aaa	2aaa		
n 1A	1AA	2Aa		
n 2a	2Aa	4aa		

$2x + 1$ = 2AAa + 5Aaa + 2aaa
$2x$ = 1AA + 4Aa + 4aa
Total = 1(1AA) : 11 (2AAa + 4Aa + 5Aaa) : 6 (2aaa + 4aa)

Fig. 3. Diagrammatic presentation of random chromosome segregation showing trisomic segregation of simplex F_1 (Aaa).

chromosome interchanges and their F_1 hybrids with *G. max* had about 50% pollen and ovule sterility. Some *G. soja* accessions are different from *G. max* by a paracentric inversion (Ahmad et al., 1977).

Apparently, the F_1 hybrids of *G. max* and *G. soja* genotypes, which differentiated either by a reciprocal translocation or by a paracentric inversion, would certainly undergo some structural changes in chromosomes. Palmer et al. (1987) suggested that the F_1 plants of *G. max* and *G. soja* with a heterozygous translocation have equally frequent alternate and adjacent chromosome segregation. A heterozygous paracentric inversion in the F_1 plants of *G. max* and *G. soja* typically results in a chromatin bridge and an acentric chromosome fragment at anaphase I of meiosis (Ahmad et al., 1977). It is highly possible that some structural changes of chromosomes such as a deletion and duplication may cause the abortion or preferential fertilization of specific gamete genotypes, thus causing distorted segregation ratios. It was interesting that the distorted trisomic segregation of the four SSR markers all fit to a ratio (1:11:6) of a simplex (Aaa) F_1 genotype. Although a change of a trisomic locus to a disomic locus is almost impossible, a deletion of a chromosome segment with a trisomic locus could occur in the interspecific F_1 hybrids. If a chromosome deletion occurs specifically on the chromosomes from *G. max*, it would decrease the frequency of the trisomic type for the marker loci on the deleted chromosome regions.

In our study, we used two *G. soja* accessions (PI 407287 and PI 81762) for developing F_2 populations. Singh and Hymowitz (1988) reported that the F_1 hybrids of PI 81762 crossed with two soybean cultivars ('Bonus' and 'Essex') had a quadrivalent in a majority of microsporocytes and had pollen fertility ranging from 49.2 to 53.3%, suggesting that PI 81762 contains a reciprocal translocation. The chromosome structure in PI 407287 has not been investigated yet. Since the four SSR markers with distorted trisomic segregation occurred in the PI 407287-derived trisomic F_2 populations, it will be necessary to examine the chromosome structure of PI 407287 to determine the causes of the distorted segregation. Palmer et al. (1987) reported that some *G. soja* accessions do carry the standard chromosome structure, and they suggested that these accessions might have resulted from introgression between various genotypes. Thus, *G. soja* genotypes with the standard chromosome structure should still possess distinct genomic differentiation from *G. max.* The genomic divergence between *G. max* and *G. soja* most likely is the major contributor to the distorted trisomic ratios in our study.

The causes of distorted trisomic segregation in the SSR markers (i.e., Satt565 and SOYGPATR) that exhibited a distorted trisomic ratio (1:11:6) in one population and a normal trisomic ratio (6:11:1) in another population (Fig. 1) are more difficult to explain. These markers also segregated in normal disomic fashion (1:2:1) in a disomic F_1 ($2n$ = 40) derived population (Fig. 1). Such different segregation ratios of the markers in different populations might indicate heterozygosity and heterogeneity for chromosome structure types (i.e.,

normal and translocation) in *G. soja* accession. Palmer et al. (1987) identified two *G. soja* accessions that consisted of two chromosome structure types. Since the F_1 hybrids for generating respective F_2 populations were not cytologically examined, the chromosome structure types and presence of heterozygosity and heterogeneity for chromosome structure types in PI 407287 remain unknown.

The numerically unbalanced genome in the primary trisomics could also be considered as a contributor to the distorted trisomic ratios. Aneuploidy is widely used in plants and is the most commonly identified chromosome abnormality in humans (Hassold et al., 1996). However, the effects of aneuploidy on gene expression and genome stability are not yet fully understood. Studies have demonstrated that genome instability can be triggered by a change in chromosome number arising from either whole genome duplications (polyploidy) or loss/gain of individual chromosomes (aneuploidy) (Matzke et al., 1999). Matzke et al. (1994) reported an aneuploid transgenic tobacco line in which the transgene complex was carried on the chromosome that was present in triplicate or quadruplicate. Although aneuploidy could explain some of the unusual inheritance, it still could not fully account for the aberrant segregation of the transgene locus. Therefore, it was postulated that this locus was structurally unstable and susceptible to deletions. Subsequent cytogenetic and molecular analyses supported this hypothesis that both large and small scale chromosomal changes occurred in the trisomics that generated the rearrangements (Papp et al., 1996). Primary trisomics in our study might have also triggered genome instability, causing unexpected trisomic inheritance. Particularly, either a reciprocal translocation or a paracentric inversion in trisomic condition might be more vulnerable for structural changes of chromosome than in the disomic state.

We found SSR markers that showed segregation distortion are located at the distal parts of MLG C1 and MLG F, and around the middle of MLG D2. The frequency of genomic changes, such as nonhomologous recombinations, can be increased in such regions. These regions could conceivably have contributed to the physical instability. In a study to verify the authenticity of a set of inter-varietal wheat chromosome substitution lines, using SSR marker, Pestsova et al. (2000) found three substituted chromosomes carried several markers specific to the donor chromosome and one "incorrect" marker specific to the recipient chromosome. All "incorrect" markers occurred in the distal region of the chromosomes. In transgenic studies, the telomeric locations could have partially accounted for the abnormal segregation ratios of the transgenic locus (Matzke et al., 1994; Papp et al., 1996; Singh, 2003).

The current study highlights the value of molecular markers for characterizing primary trisomics and assigning linkage groups to soybean chromosomes. To our knowledge, this is the first report of segregation distortion of molecular markers in primary trisomic-derived populations. If we had ignored or discarded the markers with the distorted trisomic ratios without examining other populations or other markers, we could have missed the association between certain linkage groups and chromosomes. Our results suggest that both normal and distorted trisomic segregation ratios should be considered in analyzing the association between chromosomes and linkage groups using primary trisomic and molecular marker analysis.

REFERENCES

Ahmad, Q.N., E.J. Britten, and D.E. Byth. 1977. Inversion bridges and meiotic behavior in species hybrids of soybeans. J. Hered. 68:360–364.

Blakeslee, A.F. 1921. Types of mutations and their possible significance in evolution. Am. Nat. 55:254–267.

Cregan, P.B., T. Jarvik, A.L. Bush, R.C. Shoemaker, K.G. Lark, A.L. Kahler, N. Kaya, T.T. VanToai, D.G. Lones, J. Chung, and J.E. Specht. 1999. An integrated genetic linkage map of the soybean genome. Crop Sci. 39:1464–1490.

Cregan, P.B., K.P. Kollipara, S.J. Xu, R.J. Singh, and T. Hymowitz. 2001. Primary trisomics and SSR markers as tools to associate chromosomes with linkage groups in soybean. Crop Sci. 41:1262–1267.

Faris, J.D., B. Laddomada, and B.S. Gill. 1998. Molecular mapping of segregation distortion loci in *Aegilops tauschii*. Genetics 149: 319–327.

Gardner, M.E., T. Hymowitz, S.J. Xu, and G.L. Hartman. 2001. Physical map location of the *Rps1-k* allele in soybean. Crop Sci. 41:1435–1438.

Hadley, H.H., and T. Hymowitz. 1973. Speciation and Cytogenetics. p. 97–116. *In* B.E. Caldwell (ed.) Soybeans, improvement, production, and uses. Agron. Monogr. 16, ASA, Madison, WI.

Hassold, T., M. Abruzzo, K. Adkins, D. Griffin, M. Merrill, E. Millie, D. Saker, J. Shen, and M. Zaragoza. 1996. Human aneuploidy: Incidence, origin and etiology. Environ. Mol. Mutagen. 28:167–175.

Hedges, B.R., and R.G. Palmer. 1991. Tests of linkage of isozyme loci with five primary trisomics in soybean. J. Hered. 82:491–496.

Heun, M., A.E. Kennedy, J.A. Anderson, N.L.V. Lapitan, M.E. Sorrells, and S.D. Tanksley. 1991. Construction of a restriction fragment length map for barley (*Hordeum vulgare*). Genome 34:437–447.

Honeycutt, R.J., K.E. Newhouse, and R.G. Palmer. 1990. Inheritance and linkage studies of a variegated leaf mutant in soybean. J. Hered. 81:123–126.

Khush, G.S., R.J. Singh, S.C. Sur, and A.L. Librojo. 1984. Primary trisomics of rice: Origin, morphology, cytology and use in linkage mapping. Genetics 107:141–163.

Lyttle, T.W. 1991. Segregation distorters. Annu. Rev. Genet. 25:511–557.

Matzke, M.A., E.A. Moscone, Y.D. Park, I. Papp, H. Oberkofler, F. Nerhuber, and A.J.M. Matzke. 1994. Inheritance and expression of a transgene insert in an aneuploid tobacco line. Mol. Gen. Genet. 244:219–229.

Matzke, M.A., O.M. Scheid, and A.J.M. Matzke. 1999. Rapid structural and epigenetic changes in polyploid and aneuploid genomes. Bioessays 21:761–767.

Palmer, R.G. 1976. Chromosome transmission and morphology of three primary trisomics in soybeans (*Glycine max*). Can. J. Genet. Cytol. 18:131–140.

Palmer, R.G., K.E. Newhouse, R.A. Graybosch, and X. Delanny. 1987. Chromosome structure of the wild soybean accessions from China and the Soviet Union of *Glycine soja* Sieb. & Zucc. J. Hered. 78:243–247.

Paterson, A.H., E.S. Lander, J.D. Hewitt, S. Peterson, S.E. Lincoln, and S.D. Tanksley. 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726.

Papp, I., V.A. Iglesias, E.A. Moscone, S. Michalowski, S. Spike, Y.D. Park, M.A. Matzke, and A.J.M. Matzke. 1996. Structural instability of a transgene locus in tobacco is associated with aneuploidy. Plant J. 10:469–478.

Pestsova, E., E. Salina, A. Borner, V. Korzun, O.I. Maystrenko, and M.S. Röder. 2000. Microsatellites confirm the authenticity of intervarietal chromosome substitution lines of wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 101:95–99.

Rhoades, M.M., and B. McClintock. 1935. The cytogenetics of maize. Bot. Rev. 1:292–325.

Rick, C.M., and D.W. Barton. 1954. Cytological and genetical identification of the primary trisomics of the tomato. Genetics 39:640–666.

Singh, R.J. 2003. Plant cytogenetics. 2nd ed. CRC Press, Inc., Boca Raton, Florida.

Singh, R.J., and T. Hymowitz. 1988. The genomic relationship between *Glycine max* (L.) Merr. and *G. soja* Sieb. and Zucc. as revealed by pachytene chromosome analysis. Theor. Appl. Genet. 76:705–711.

Tsuchiya, T. 1967. Establishment of a trisomic series in a two-rowed cultivated variety of barley. Can. J. Genet. Cytol. 9:667–682.

Walbot, V. 1988. Preparation of DNA from single rice seedlings. Rice Genet. Newsl. 5:149–151.

Webb, D.M., B.M. Baltazar, A.P. Rao Arelli, J. Schupp, K. Clayton, P. Keim, and W.D. Beavis. 1995. Genetic mapping of soybean cyst nematode race-3 resistance loci in the soybean PI 437654. Theor. Appl. Genet. 91:574–581.

Xu, S.J., R.J. Singh, K.P. Kollipara, and T. Hymowitz. 2000. Primary trisomics in soybean: Origin, identification, breeding behavior, and uses in gene mapping. Crop Sci. 40:1543–1551.

Xu, Y.B., L.H. Zhu, J.H. Xiao, N. Huang, and S.R. McCouch. 1997. Chromosomal regions associated with segregation distortion of molecular markers in F_2, backcross, doubled haploid, and recombinant inbred populations of rice (*Oryza sativa* L.). Mol. Gen. Genet. 253:535–545.

Yamanaka, N., S. Ninomiya, M. Hoshi, Y. Tsubokura, M. Yano, Y. Nagamura, T. Sasaki, and K. Harada. 2001. An informative linkage map of soybean reveals QTLs for flowering time, leaflet morphology and regions of segregation distortion. DNA Res. 8:61–72.

Zou, J.J., R.J. Singh, and T. Hymowitz. 2003a. Association of the yellow leaf (*y10*) mutant to soybean chromosome 3. J. Hered. 94:352–354.

Zou, J.J., R.J. Singh, J. Lee, S.J. Xu, P.B. Cregan, and T. Hymowitz. 2003b. Assignment of molecular linkage groups to soybean chromosomes by primary trisomics. Theor. Appl. Genet. 107:745–750.